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Cardiovascular Risk Factors and Dehydroepiandrosterone Sulfate Among Latinos in the Boston Puerto Rican Health Study

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Low blood dehydroepiandrosterone sulfate (DHEAS) levels have strong positive associations with stroke and coronary heart disease. However, it is unclear whether DHEAS is independently associated with cardiovascular risk factors. Therefore, we examined the association between cardiovascular risk factors and DHEAS concentration among a high-risk population of Latinos (Puerto Ricans aged 45 to 75 years at baseline) in a cross-sectional analysis of the Boston Puerto Rican Health Study. Of eligible participants, 72% completed baseline interviews and provided blood samples. Complete data were available for 1355 participants. Associations between cardiovascular risk factors (age, sex, total cholesterol, high-density lipoprotein cholesterol, triglycerides, and glucose) and log-transformed DHEAS ($\mu\text{g/dL}$) were assessed. In robust multivariable regression analyses, DHEAS was significantly inversely associated with age ($\beta = -12.4$; 95% CI: $-15.2, -9.7$; per 5 years), being female (vs. male) ($\beta = -46$; 95% CI: $-55.3, -36.6$), and plasma triglyceride concentration ($\beta = -0.2$; 95% CI: $-0.3, -0.1$; per 10 mg/dL) and was positively associated with total cholesterol and plasma glucose levels ($\beta = 1.8$; 95% CI: 0.6, 3 and $\beta = 0.2$; 95% CI: 0.04, 0.3, respectively, per 10 mg/dL) after adjustment for smoking, alcohol, and physical activity and for postmenopausal hormone use in women. Estimates were unchanged after adjustment for measures of chronic disease and inflammation. Women exhibited a stronger age-related decline in DHEAS and a positive association with glucose in contrast to findings among men ($P_{\text{interaction}} < 0.05$). In conclusion, in this large study of Latinos with a heavy cardiovascular risk factor burden, we observed significant associations between cardiovascular disease (CVD) risk factors and DHEAS, with variations by sex. These findings improve our understanding of the role DHEAS may play in CVD etiology.

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Puerto Ricans have the highest cardiovascular risk factor profile of US Latino heritage groups, suggesting an elevated risk of future cardiovascular disease (CVD) morbidity and mortality [1]. Results from the Hispanic Community Health Study/Study of Latinos indicated

Abbreviations: BMI, body mass index; BPHS, Boston Puerto Rican Health Study; CRP, high-sensitivity C-reactive protein; CVD, cardiovascular disease; DBP, diastolic blood pressure; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; HbA_{1c}, glycosylated hemoglobin; HDL-C, high-density lipoprotein cholesterol; SBP, systolic blood pressure; TG, triglyceride; VLDL, very-low-density lipoprotein.

that 25% of Puerto Ricans had three or more metabolic cardiovascular risk factors. For example, 16% of men and 19% of women had diabetes, 41% of men and 51% of women were obese, and 27% of men and 29% of women had hypertension. The Boston Puerto Rican Health Study (BPHS) provides a unique opportunity to study dehydroepiandrosterone sulfate (DHEAS) because of its detailed physiologic data on a large cohort of Latinos with high cardiovascular risk. The purpose of this study was to examine the association between DHEAS concentration and cardiovascular risk factors among Puerto Rican adults to better understand the higher burden of CVD among Puerto Ricans.

DHEAS, produced primarily in the adrenal cortex, is the most abundant circulating hormone in humans. Evidence suggests that low DHEAS level may be a risk factor for total and cause-specific CVD, such as coronary heart disease [2–4] and stroke [3, 5]. Moreover, low DHEAS level has been associated with a greater prevalence and incidence of cardiometabolic risk factors (high blood pressure, insulin resistance, and impaired fasting glucose level), which are highly prevalent among particular Latino heritage groups such as Puerto Ricans [6–9].

Animal and human models support a mechanistic role for DHEAS in CVD pathophysiology through its influence on vasodilation and blood pressure [10, 11], glucose metabolism [12, 13], triglyceride (TG) concentration, and endothelial dysfunction [14]. However, with few exceptions, associations between cardiovascular risk factors and DHEAS have generally been examined in studies with statistical adjustment limited to demographic factors (*i.e.*, sex and age) [15–18]. Furthermore, DHEAS may vary by race/ethnicity [6–9]; however, few data are available for Latinos [6, 7]. Moreover, we are unaware of any studies that have evaluated associations between cardiovascular risk factors and DHEAS concentration among Latinos. Therefore, we hypothesized that adverse cardiovascular risk factors would be associated with lower DHEAS concentration.

1. Materials and Methods

A. The BPHS

The BPHS is an ongoing prospective cohort study initiated to examine the association between psychosocial stress, allostatic load, and systemic health (*e.g.*, depression, disability, cognitive impairment, and metabolic conditions). Details of the study design were previously described in detail [19]. Briefly, 1500 self-identified Puerto Ricans aged 45 to 75 years who were living in the Boston, Massachusetts, metropolitan area and were able to answer questions in either English or Spanish were enrolled between 2004 and 2009. Participants were recruited using door-to-door enumeration based on census tract data and community outreach. All data were collected through in-home assessments, consisting of interviewer-administered questionnaires and examinations by trained bilingual staff. Participants were compensated \$50 for their time. Baseline questionnaires included questions on demographic, socioeconomic, psychosocial and behavioral factors, medical history, cognitive function, physical disability, medication use, diet, and acculturation. Anthropometric and blood pressure measurements were obtained during the interview process, with biomarker samples obtained the following day in the morning or as soon as possible thereafter. Follow-up assessments were conducted 2 and 5 years later; however, the analyses presented herein are restricted to baseline data [19]. The analytic sample included 1450 participants with baseline DHEAS measurement.

B. Blood Sample Assay

Detailed information on blood sample assays was previously described [19]. Briefly, 12-hour fasting blood draws were conducted in the home by a certified phlebotomist on the morning of the day following the home interview. On average, blood samples were collected at 08:18 ± 1.23 hours (median = 08:15 hours; interquartile range, 07:15 to 09:22 hours). Blood samples

were carried back to the Nutrition Evaluation Laboratory at the Human Nutrition Research Center on Aging at Tufts University (Boston, MA) on the day of collection in coolers equipped with dry ice and were inventoried according to standard protocol. Samples were immediately cooled to 4°C, and plasma and serum were separated within 4 hours in a refrigerated centrifuge. Aliquots were saved in 1-mL cryogenic, screw-cap tubes and stored at –70°C for later processing.

Serum DHEAS concentration (μg/dL) was measured by solid-phase two-site chemiluminescent immunometric assay using the Immulite 1000 DHEA-S kit (LKDS1; Siemens Medical Solutions Diagnostics, Los Angeles, CA) [20], with a mean intra-assay coefficient of variation of 6.8% to 9.5%. Total cholesterol, high-density lipoprotein cholesterol (HDL-C), and TGs were analyzed from EDTA plasma using standard laboratory assays [19]. Low-density lipoprotein cholesterol and very-low-density lipoprotein (VLDL) were calculated as $VLDL = TGs/5$; low-density lipoprotein cholesterol = total cholesterol – (VLDL + high-density lipoprotein). Serum high-sensitivity C-reactive protein (CRP) and insulin levels were measured with a solid-phase, two-site chemiluminescent immunometric assay (Immulite 1000 High Sensitive CRP Kit [21] and Insulin Kit [22], respectively; Siemens Medical Solutions Diagnostics). Serum glucose level was measured by enzymatic, kinetic reaction with Olympus Glucose Reagents (OSCR6121; Olympus America Inc., Melville, NY). Glycosylated hemoglobin (HbA_{1c}) level was estimated by determining the ratio of HbA_{1c} to Hb along with a conversion factor and analyzing whole blood hemolysate by latex-enhanced immunoturbidimetric to determine the HbA_{1c} value. Intra-assay coefficients of variation for total cholesterol, HDL-C, TGs, and glucose were <5%, and they were <10% for CRP (4.2% to 6.4%) and insulin (5.2% to 6.4%).

C. Cardiovascular Risk Factors

Data on cardiovascular risk factor profiles were obtained from baseline interviewer-administered questionnaires, clinical measurements, and blood samples. The baseline questionnaire included demographic (sex, age), lifestyle and behavioral factors, history of chronic disease, and medication use. The questionnaire was based on national surveys validated in both Hispanic/Latino and elderly populations (National Health and Nutrition Examination Survey III, the Hispanic Health and Nutrition Examination Survey, and the National Health Interview Survey's Supplement on Aging) [19]. Self-reported history of CVD (yes/no) and diabetes (yes/no) and frequency and history of alcohol use (g/d) and smoking status (never/former/current) were collected. Current physical activity was assessed using a modified Paffenbarger questionnaire, which was previously tested in an elderly Puerto Rican population [23, 24]. All anthropometric measures were taken in duplicate by trained interviewers and were averaged for analysis. Weight was measured using a clinical scale (Toledo Weight Plate, Model I5S; Bay State and Systems Inc., Burlington, MA), and height was measured with a portable stadiometer. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) levels were measured in duplicate using an electronic sphygmomanometer (Dinamap™ Model 8260; Critikon, Tampa, FL) at three different time points after short rests with the participant seated. Blood pressure measurements were based on the mean of the second and third measurements.

D. Statistical Analysis

Descriptive analyses comparing the distribution of cardiovascular risk factors by sex were conducted using Pearson χ^2 statistic for categorical variables and Student *t* test for normally distributed variables. A Wilcoxon rank sum test was used to test the distribution of non-normally distributed variables across sexes. Deviations from normality were assessed visually by boxplots and statistically by the Shapiro-Wilk test. Partial Spearman correlation coefficients, adjusted for age and sex, were estimated between DHEAS concentration (μg/dL) and continuous cardiovascular risk factors.

Candidate cardiovascular risk factors included age (years), sex, history of heart disease (yes/no) and diabetes (yes/no), SBP (mm Hg), DBP (mm Hg), total cholesterol (mg/dL), HDL-C (mg/dL), TGs (mg/dL), glucose (mg/dL), and CRP (mg/L). Cardiovascular risk factors associated with DHEAS concentration ($\mu\text{g/dL}$; dependent variable) were identified using automated stepwise linear regression with an entry criterion of $\alpha = 0.20$ and an exit criterion of $\alpha = 0.10$, with the forced inclusion of age and sex [25]. For the linear regression analysis, DHEAS ($\mu\text{g/dL}$), glucose (mg/dL), and TG (mg/dL) concentrations were log transformed to achieve normality because of their highly skewed distributions. The associations between each of the selected cardiovascular risk factors and DHEAS concentration were modeled using robust multivariable linear regression models, which provide estimates resistant to outliers.

Three nested multivariable models, adjusted for groups of related potential confounders and intermediates and selected on the basis of prior well-established research, were estimated in the total population and separately by sex. Model 1 was mutually adjusted for the candidate predictors identified by the stepwise regression modeling procedure, using the entry and exit criteria previously described. Model 2, the primary model, was additionally adjusted for potential confounders related to lifestyle/behavioral factors: smoking status (never, former, current), alcohol consumption (g/d), physical activity metabolic equivalents per week, and postmenopausal hormone use among women (yes/no). Model 3 further included history of chronic disease and inflammation [history of diabetes (yes/no), body mass index (BMI; kg/m^2), antihypertensive medication use (yes/no), lipid-lowering medication use (yes/no), SBP (mm Hg), and CRP (mg/L)]. Parameter estimates (β) with robust SE and 95% CIs were presented (robust regression modeling allows for estimates robust to extreme observations and modeling assumptions [22]).

On the basis of prior research, we hypothesized *a priori* that DHEAS concentration differed significantly by sex, being higher among men than among women. Therefore, we proposed to evaluate the effect modification of cardiovascular risk factors and DHEAS concentration by sex. Significance of the interaction was assessed using a Wald test statistic for the interaction term. Analyses were restricted to participants with full data for all covariates; however, <10% of observations were missing data on any one covariate, thus eliminating missingness as a source of confounding in our analyses. The covariates with the greatest missingness were SBP and DBP, with 2% missing data. All *P* values were two-sided with $\alpha = 0.05$. Analyses were conducted with SAS (version 9.2; SAS Institute, Cary, NC).

E. Statement of Ethics

This study was approved by the institutional review boards of Tufts Medical Center and Northeastern University, and all procedures followed were in accordance with institutional guidelines. Participants provided written informed consent to participate [19].

2. Results

Among the 1355 participants with full covariate data, the mean age was 57 years in both men and women. As expected, DHEAS concentration varied by sex, with median concentration lower among women than among men (Table 1). Moreover, the distribution of cardiovascular risk factors also varied significantly by sex (Table 1). Women exhibited higher BMI, total cholesterol and HDL-C levels, and CRP concentration >3 mg/L ($P < 0.001$) than men. Men displayed higher SBP and DBP levels ($P < 0.01$) and were more likely to report current smoking than were women ($P < 0.001$). Partial Spearman correlations adjusted for age and sex showed positive linear associations between DHEAS concentration and total cholesterol level, low-density lipoprotein level, physical activity, and alcohol consumption ($P < 0.05$) (Table 2). In contrast, DHEAS concentration was inversely correlated with insulin and BMI ($P < 0.01$).

In univariate analyses, DHEAS concentration varied significantly across cardiovascular risk factors in both men and women (Fig. 1). Among men, median concentration of DHEAS did not vary significantly across CVD risk factors, with one exception. The median

Table 1. Distribution of Cardiovascular Disease Risk Factors in Puerto Rican Adults by Sex

Characteristics	Overall	Males	Females	P Value
	n = 1355	n = 400	n = 955	
Age, y	57.0 ± 7.5 [56.0 (51.0–62.0)]	56.5 ± 7.8 [56.0 (51.0–62.0)]	57.1 ± 7.4 [56.0 (51.0–62.0)]	0.16 ^a
DHEAS(μg/dL)	84.6 ± 67.9 [68.0 (39.0–111.0)]	117.0 ± 84.4 [99.5 (59.0–154.5)]	71.1 ± 54.3 [58.0 (34.0–94.0)]	<0.001 ^b
CVD risk factors				
BMI, kg/m ²	31.7 ± 6.4 [30.9 (27.3–35.2)]	29.6 ± 5.3 [29.2 (26.2–33.0)]	32.5 ± 6.7 [31.8 (28.0–36.5)]	<0.001 ^a
Systolic blood pressure, mm Hg	135.0 ± 19.2 [133.3 (121.3–146.0)]	137.4 ± 19.1 [135.6 (124.9–148.3)]	134.0 ± 19.2 [132.5 (119.8–145.5)]	0.003 ^a
Diastolic blood pressure, mm Hg	81.1 ± 10.7 [80.5 (73.5–87.8)]	83.0 ± 11.1 [82.4 (75.3–89.0)]	80.2 ± 10.4 [79.8 (73.0–87.0)]	<0.001 ^a
Total cholesterol, mg/dL	184.4 ± 41.8 [182.0 (154.0–212.0)]	173.9 ± 43.0 [172.0 (143.0–202.0)]	188.8 ± 40.5 [188.0 (159.0–216.0)]	<0.001 ^a
HDL-C, mg/dL	45.1 ± 12.5 [43.0 (37.0–51.0)]	40.5 ± 12.3 [38.0 (32.0–47.0)]	47.1 ± 12.1 [45.0 (39.0–53.0)]	<0.001 ^b
Triglycerides, mg/dL	163.2 ± 114.4 [136.0 (100.0–191.0)]	175.5 ± 143.2 [144.0 (98.5–202.5)]	158.0 ± 99.5 [134.0 (100.0–185.0)]	0.12 ^b
Fasting plasma glucose, mg/dL	119.4 ± 50.8 [102.0 (92.0–127.0)]	121.2 ± 53.7 [102.0 (92.0–129.0)]	118.6 ± 49.5 [102.0 (99.0–126.0)]	0.72 ^b
HbA _{1c} ≥6, %	70.6	67.5	71.7	0.14 ^c
CRP ≥3, mg/L, %	56.3	42.8	62.0	<0.0001 ^c
History of CVD, %	20.4	22.5	19.6	0.22 ^c
Hypertension, % ^d	67.5	68.8	66.9	0.51 ^c
Diabetes, %	38.5	40.3	37.8	0.40 ^c
Antihypertensive medication, %	58.8	58.8	58.7	>0.99 ^c
Lipid-lowering medication, %	40.2	38.8	40.7	0.50 ^c
Lifestyle factors				
Current smoker, %	24.7	33.3	21.1	<0.0001 ^c
Alcohol, g/d	1.5 ± 0.6 [1.0 (1.0–2.0)]	1.7 ± 0.7 [2.0 (1.0–2.0)]	1.4 ± 0.6 [1.0 (1.0–2.0)]	<0.0001 ^b
Physical activity, METs/wk	31.6 ± 4.0 [30.2 (28.2–33.0)]	32.5 ± 5.7 [31.1 (28.6–32.1)]	31.2 ± 4.0 [30.2 (28.3–33.0)]	0.001 ^b

Values are means ± SD, [medians (IQR)], or percentages.

Abbreviation: METs/wk, metabolic equivalents per week.

^aP value based on two independent sample *t* tests.

^bP value based on Wilcoxon rank sum test.

^cP value based on χ^2 test.

^dBased on values or antihypertensive medication use.

concentration of DHEAS among men with total cholesterol level ≥ 200 mg/dL (115 μ g/dL) was significantly higher than in those with total cholesterol level < 200 mg/dL (89 μ g/dL; $P = 0.01$). In contrast, among women, median DHEAS concentration varied significantly across hypertension status and history of CVD. Median DHEAS concentration was higher among women without hypertension (69 μ g/dL) than among those with hypertension (54 μ g/dL) and among women who did not report a history of CVD (61 μ g/dL) than among those who did (46 μ g/dL; $P < 0.001$ for both comparisons).

Stepwise linear regression was used to identify cardiovascular risk factors that were independently associated with DHEAS concentration. After the forced inclusion of age and female sex, total cholesterol, high-density lipoprotein, TG, and glucose levels were selected as cardiovascular risk factors associated with DHEAS concentration for inclusion in the final multivariable models ($P < 0.1$). As expected, in sex-combined analyses using multivariable robust linear regression, age and female sex exhibited strong inverse associations with DHEAS concentration in all multivariable models (Table 3). When mutually adjusted for all

Table 2. Partial Spearman Correlations Between DHEAS Concentration ($\mu\text{g/dL}$) and Cardiovascular Risk Factors

	r^a	<i>P</i> Value
Age, y	−0.25	<0.0001
Systolic blood pressure, mm Hg	0.03	0.31
Diastolic blood pressure, mm Hg	0.03	0.32
Cholesterol, mg/dL	0.07	0.02
HDL, mg/dL	0.02	0.43
Triglycerides, mg/dL	−0.05	0.05
CRP, mg/L	0.03	0.34
Fasting glucose, mg/dL	0.05	0.06
HbA _{1c} %	0.01	0.81
BMI, kg/m ²	−0.08	0.01
Physical activity, METs/wk	0.07	0.01
Alcohol, g/d	0.11	0.0001

Abbreviations: HDL, high-density lipoprotein; METs/wk, metabolic equivalents per week.

^aPartial Spearman correlation coefficient adjusted for age and sex, except for age, which is adjusted only by sex.

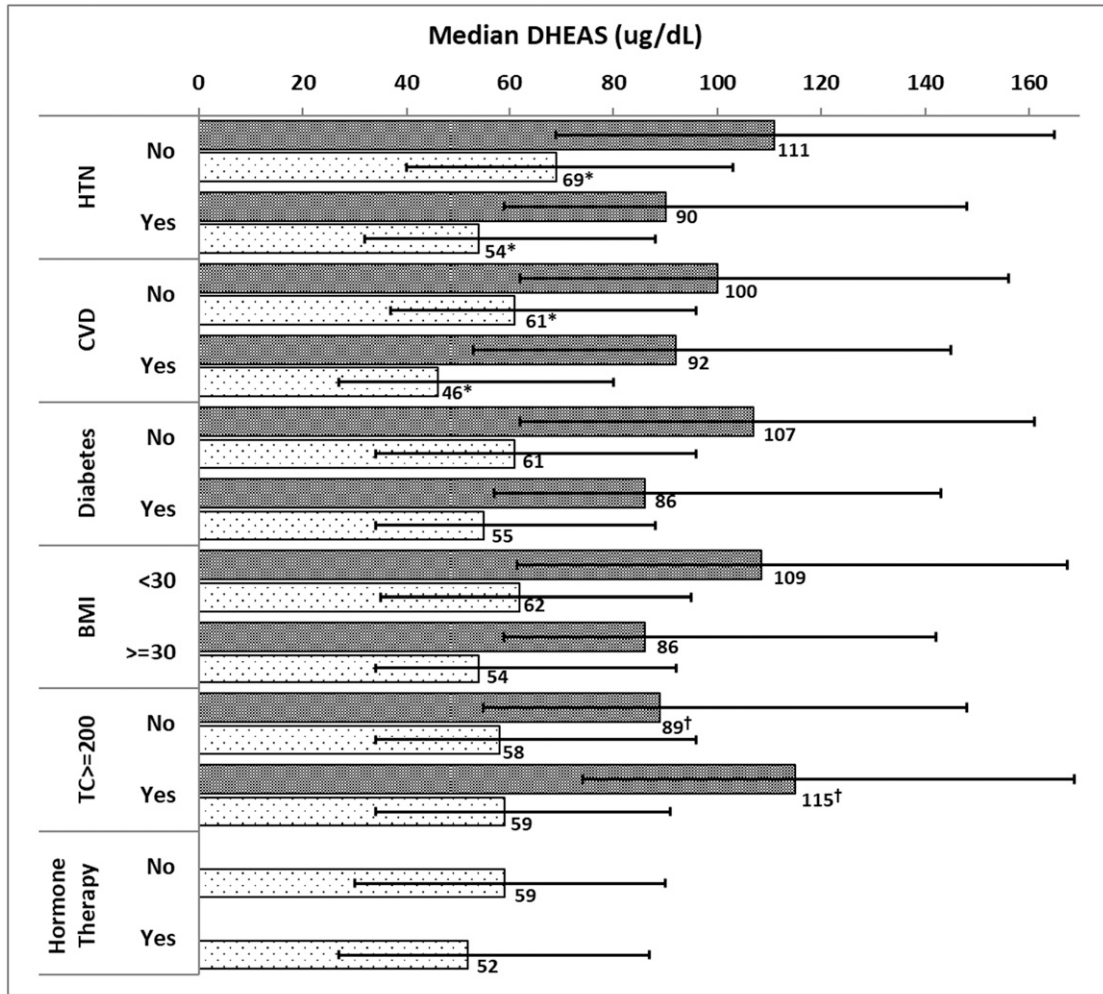
predictors (model 1), DHEAS concentration was 1.9% (95% CI: 0.7, 3.1) higher with each 10-mg/dL increase in total cholesterol level and 0.2% (95% CI: 0.03, 0.3) higher with each 10% increase in glucose level. In contrast, a 0.2% lower DHEAS concentration (95% CI: −0.3, −0.1) was seen with each 10% increase in TG level. Estimates were modestly attenuated toward the null after adjustment for potential lifestyle/behavioral confounders (model 2). After adjustments for potential history of chronic disease and inflammation (model 3), glucose level was no longer significantly associated with DHEAS concentration.

Evidence suggests that the association between cardiovascular risk factors and DHEAS concentration may vary by sex ($P_{\text{interaction}} < 0.05$) (Table 3). Age was associated with significantly lower DHEAS concentration among women than among men (14.7% vs 6.9%, per 5-year increase; $P_{\text{interaction}} = 0.02$). Glucose level was associated with significantly greater DHEAS concentration ($\mu\text{g/dL}$) among women but not among men ($P_{\text{interaction}} = 0.03$).

3. Discussion

In this unique study in a large sample of Latinos at high risk of CVD, serum DHEAS levels exhibited significant associations with age, sex, and lipid and glucose concentrations. As expected, the strongest associations were observed with age and sex. We observed paradoxical support for the role of DHEAS in cardiovascular risk. Significant associations between plasma lipids, glucose, and serum DHEAS levels were independent of potential confounders, history of chronic CVD, and inflammation. However, the direction of the associations varied across cardiovascular risk factors and by sex. Specifically, higher DHEAS concentration was associated with higher total cholesterol concentration among men, whereas higher DHEAS concentration was associated with higher glucose concentration among women. In contrast, higher DHEAS concentration was associated with lower TG levels among both men and women. Hence, the role of DHEAS in cardiovascular risk remains uncertain because of these paradoxical and conflicting associations across cardiovascular risk factors.

Whether DHEAS concentration is influenced by race/ethnicity is uncertain because of a paucity of adrenal hormonal data available for racial/ethnic minorities [6–9]. Some studies have reported lower DHEAS concentrations among black women [8] and Japanese men [26] compared with their non-Hispanic white counterparts, whereas others have reported null findings [6, 7, 9]. Only two studies included Latinos, and both failed to find significant variation by race/ethnicity; however, specific information regarding heritage background was not provided [6, 7]. Of note, the distribution of DHEAS concentration in this study was similar to that reported by others with similarly aged populations [11, 27].



Sample size	HTN		CVD		Diabetes		BMI		TC _≥ 200 mg/dL		Hormone therapy	
	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes
Men	125	275	310	90	239	161	224	176	294	106	-	-
Women	316	639	768	187	594	361	371	584	575	380	931	24

Figure 1. Sex-specific differences in median DHEAS concentration ($\mu\text{g/dL}$) by cardiovascular risk factors. Median DHEAS concentration for men is presented by solid bars and for women by dotted bars. Bars reflect bounds of 95% CI. *P* values are for sex-specific differences in median DHEAS concentration between the presence or absence of specified cardiovascular risk factors. **P* < 0.001; †*P* = 0.01. HTN, hypertension; TC, total cholesterol.

DHEAS exhibited a stronger age-related decline among women than among men, which raises questions about whether DHEAS may be more strongly related to female than to male cardiovascular health. Although it is well established that DHEAS concentration declines with age, it is less understood whether age-related declines vary by sex. We observed a stronger inverse association between DHEAS concentration and age among women than among men ($P_{\text{interaction}} = 0.02$), consistent with reports in short-term follow-up studies of change in DHEAS concentration by sex [27]. However, no differences by sex were reported in another study tracking individuals over 5 years [28]. Therefore, our results may simply reflect a lower baseline concentration of DHEAS among women.

The overall findings in this study contrasted with previous work in both animal and human models, which supports potential mechanisms by which DHEAS concentration may influence the pathogenesis of CVD through cardiovascular risk factors. Previous studies suggested that

Table 3. Multivariable Associations Between Cardiovascular Risk Factors and DHEAS ($\mu\text{g/dL}$)

Cardiovascular Risk Factors	Model 1	Model 2	Model 3
Total	β (95% CI)	β (95% CI)	β (95% CI)
Age, y ^a	-13.9 (-16.6, -11.2)	-12.4 (-15.2, -9.7)	-12.9 (-15.9, -9.9)
Female sex ^b	-51.3 (-60.4, -42.3)	-46.0 (-55.3, -36.6)	-43.7 (-53.3, -34.2)
Cholesterol, mg/dL ^c	1.9 (0.7, 3.1)	1.8 (0.6, 3)	1.6 (0.4, 2.8)
HDL, mg/dL	-0.2 (-0.6, 0.2)	-0.3 (-0.2, 0.7)	-0.4 (-0.8, 0.04)
Triglycerides, mg/dL ^d	-0.2 (-0.3, -0.1)	-0.2 (-0.3, -0.1)	-0.2 (-0.3, -0.1)
Glucose, mg/dL ^d	0.2 (0.03, 0.3)	0.2 (0.04, 0.3)	0.2 (-0.02, 0.3)
Sex Stratified	Men ^e	Women ^e	P Sex Interaction
Age, y ^a	-6.9 (-11.7, -2.1)	-14.7 (-18.1, -11.4)	0.02
Cholesterol, mg/dL ^c	3.2 (1.0, 5.3)	1.2 (-0.2, 3.6)	0.17
HDL, mg/dL	0.2 (-0.6, 1.0)	-0.6 (-1.1, -0.1)	0.15
Triglycerides, mg/dL ^c	-0.1 (-0.3, 0.1)	-0.2 (-0.3, -0.1)	0.25
Glucose, mg/dL ^c	-0.04 (-0.3, 0.2)	0.3 (0.1, 0.4)	0.03

Multivariable analyses were conducted using robust linear regression between selected cardiovascular risk factors and DHEAS. DHEAS, triglycerides, and glucose levels were log transformed to achieve normality. Model 1 mutually adjusted for all selected predictors. Model 2: Model 1 + smoking, alcohol, physical activity, and among women postmenopausal hormone use. Model 3: Model 2 + diabetes status, history of cardiovascular disease, body mass index, antihypertensive medication use, lipid-lowering medication use, systolic blood pressure, and C-reactive protein. Bold face indicates results are statistically significant $\alpha = 0.05$.

Abbreviation: HDL, high-density lipoprotein.

^aPercentage change in DHEAS per 5-year increase in age.

^bPercentage change in DHEAS per unit increase in predictor (e.g., women compared with men).

^cPercentage change in DHEAS per 10 mg/dL increase in total cholesterol.

^dPercentage change in DHEAS per 10% change in each predictor.

^eSex-specific models adjusted for covariates in Model 2.

DHEAS may act through atherosclerotic-related mechanisms, such as inhibiting the migration and proliferation of cells within the vascular wall and increasing vascular smooth muscle cell apoptosis, thereby reducing vascular remodeling after injury [29]. Moreover, DHEAS has been suggested to improve insulin resistance through reduced hepatocyte glucose production [12] and improved peripheral tissue glucose clearance. However, our findings do not support an inverse association between DHEAS and glucose concentrations [13]. Of note, it remains uncertain whether DHEAS acts alone or predominantly through conversion to estrogens/androgens. Moreover, although DHEAS can act independently in many cell types, including vascular endothelium cells [30], its androgenic/estrogenic activity may vary given the underlying hormonal environment [31]. Hence, apparent inconsistencies between DHEAS and cardiovascular risk factors by sex may be partially explained by hormonal differences.

In addition, it is unclear whether our disparate findings were influenced by the heavy burden of cardiovascular risk factors in this population, leading to potential differences in DHEAS activity. In fact, when median DHEAS concentrations were compared across the cardiovascular risk factor profile (Fig. 1), median DHEAS concentration was higher among those without a history of hypertension, CVD, or diabetes or a BMI ≥ 30 kg/m² than among those with these CVD risk factors; however, differences in median DHEAS concentration were significant among women only for hypertension and history of CVD. Hence, further work is needed to explore these findings in other populations.

Previous studies reported inconsistent findings with respect to the association between plasma lipids and serum DHEAS levels. We have limited our discussion to the few studies that used multivariable methods, noting that all have been conducted among postmenopausal women, limiting generalizability to men [15, 17, 18]. No multivariable associations between total cholesterol and DHEAS levels were reported among samples of middle-aged postmenopausal women from the Massachusetts Women's Health Study [15] or the Atherosclerosis

Risk in Communities Study [18]. It is worth noting that our results showed some consistency with these findings. Although the interaction with sex for total cholesterol and DHEAS levels was not statistically significant ($P_{\text{interaction}} = 0.17$), stratified analysis showed significantly higher DHEAS concentration with higher total cholesterol concentration among men ($\beta = 3.2$; 95% CI: 1.0, 5.3) but not among women ($\beta = 1.2$; 95% CI: -0.2 , 3.6). DHEAS is derived from cholesterol; therefore, the overall positive association observed with total cholesterol may be due to DHEAS biosynthesis. Our findings for TGs were similar to those demonstrated by Bell *et al.* [32], who reported lower DHEAS concentration with higher TG levels when adjusted for age, BMI, smoking, alcohol, and exercise among a population-based sample of postmenopausal Australian women. Our data provide initial multivariable-adjusted results for an association between TGs and DHEAS concentration among men.

Several small randomized trials demonstrated that dehydroepiandrosterone (DHEA) supplementation lowered glucose concentration or improved insulin sensitivity through glucose stabilization and improved insulin action [33–35], but others have been null [36–38] or have indicated that higher DHEAS concentration may be associated with hyperinsulinemia [39]. All have been limited by small sample size, short follow-up, or highly specific populations, such as the elderly or those with adrenal hormone deficiencies [33–36, 38]. We found that higher glucose level was associated with higher DHEAS concentration and observed that the association may be restricted to women ($P_{\text{interaction}} = 0.03$). Similarly, in a multiethnic population of postmenopausal women, those in the highest quartile of DHEAS concentration had 68% greater odds of impaired fasting glucose level than women in the lowest quartile (OR = 1.68; 95% CI: 1.23, 2.29; P trend < 0.001) when adjusted for age, race/ethnicity, and study site [40]. Estimates remained elevated, but were no longer significant, after adjustment for lifestyle and behavioral confounders (OR = 1.43; 95% CI: 0.97, 2.13; P trend = 0.02).

The discrepancy of these findings may be due to variation in insulin resistance; Farah *et al.* [41] demonstrated that insulin resistance was associated with increased production of DHEAS, which may partially explain our findings, given the high burden of diabetes and overweight/obesity in this study population. Moreover, the association between DHEAS concentration and adiposity has been inconsistent, with studies reporting null, positive, and inverse associations [2, 42–44]. Although a weak inverse correlation was observed between BMI and DHEAS in our study, BMI was not identified as a significant explanatory variable of DHEAS in multivariable models. This may suggest that DHEAS is more closely associated with mechanisms of metabolic regulation through pathways other than obesity.

Furthermore, chance findings associated with evaluation of multiple subgroups in our analyses may also explain the apparent heterogeneity. Further investigation is needed to examine these findings and sex differences in the association between DHEAS and glucose levels across other populations.

It is important to note that we did not observe multivariable associations between SBP or DBP and DHEAS levels, as previously reported in observational cohorts [11, 15]. However, our findings are consistent with those of a randomized controlled trial of DHEA supplementation among frail elderly patients, which failed to show significant associations with either SBP or DBP level [45]. Data from observational cohorts have generally involved populations with considerably healthier cardiovascular profiles than those in the current study or the aforementioned trial of DHEA supplementation; hence, comorbid conditions or medication use could mask an underlying association with blood pressure.

This study has several strengths and limitations. Important strengths include the large sample size of Latinos, interviewer-administered questionnaires, and clinically assessed biomarkers. DHEAS is a more stable marker than DHEA, given a longer half-life, and it does not exhibit a strong diurnal variation, which is further attenuated or even lost with increasing age [14, 46–48]. However, it should be noted that samples were collected in the morning, which does not correspond with the acrophase of the 24-hour circadian cycle of DHEAS, which is noted to occur in the late afternoon (approximately 16:00) and to be of small amplitude [46, 47, 49]. Given the age distribution of our population, the timing of blood collection is unlikely to result in

systematic bias, and a single measure of DHEAS is expected to reasonably reflect the mesor [46, 47]. In addition, we appreciate potential concerns regarding multiple testing; however, adjusted type I error would result in inflated type II error and a greater risk of failing to observe key biologic associations for future investigation [50]. These data provide evidence suggesting that DHEAS may influence cardiovascular risk through lipid and glucose mechanisms; however, it remains uncertain whether DHEAS plays a direct role in the etiologic pathogenesis of cardiovascular risk or serves as a sensitive marker of subclinical vascular disease. Therefore, further studies with longitudinal measures of DHEAS and cardiovascular risk factors are needed.

This study reported DHEAS concentrations across cardiovascular risk factors among Puerto Rican adults. However, generalizability of these results to other racial/ethnic and socio-demographic groups may be limited. Moreover, given the cross-sectional nature of the study, the temporality of the associations cannot be identified. It has been proposed that changes in DHEAS concentration over time may be a stronger predictor of cardiovascular mortality than baseline concentration; whether this is consistent with changes in cardiovascular risk factors could not be explored in the current analysis and requires further investigation.

4. Conclusions

This study in an understudied minority group with a high burden of cardiovascular risk factors provides evidence to support the association between DHEAS and key cardiovascular risk factors, primarily lipid and glucose levels. However, the role of DHEAS in influencing cardiovascular risk may be influenced by an individual's underlying cardiovascular risk burden, in some cases leading to seemingly paradoxical results. These findings may inform future research and eventually interventions in this and other high-risk populations.

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